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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:	Davin C. Dillon *et al.*
Group Art Unit:	1637
Application No:	10/010,742
Filed:	November 30, 2001
For:	COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER
Examiner:	Teresa E. Strzelecka, Ph.D.
Docket No.:	210121.491C7

DECLARATION OF DR. DAVIN DILLON, Ph.D.

Mail Stop Amendment
Commissioner for Patents
Washington, D.C. 20231

The undersigned, Dr. Davin Dillon, hereby declares:

1. I am a Scientist and Director, Product Management at Corixa Corporation, the assignee of the subject application, and a named inventor of the present invention.

2. I have reviewed the Office Action dated May 26, 2004 and in particular the rejections under 35 U.S.C. §§ 101 and 112, and am familiar with the instant application. I provide this Declaration to assist the Examiner in analyzing the claimed invention.

3. The following analyses and experiments were carried out under my supervision.

4. The specification as filed clearly states at page 103, lines 17-25 that the polynucleotide set forth in SEQ ID NO:52 was determined to be overexpressed in breast tumor tissue compared to other tissues tested by a visual analysis of microarray data. Further, using computer sequence analysis software available in the art and described in the specification, such as the DNAStar Seqman™ program, the specification states that SEQ ID NO:52 matches the two

template sequences of 228686.6 and 228686.8, the polynucleotide sequences of which are set forth in SEQ ID NOs:302 and 304. Further, using the DNAStar sequence analysis program or other commercially available sequence analysis programs, it is easily confirmed by the skilled artisan that SEQ ID NO:52 is 100% identical to the polynucleotide set forth in SEQ ID NO:305 as shown by the attached alignment in Figure 1. Based on this analysis, I conclude that SEQ ID NO:52 is a partial sequence of the polynucleotide set forth in SEQ ID NO:305 (also referred to as B854P) and, as such, the polynucleotide set forth in SEQ ID NO:305 would be expected to have the same expression profile as the polynucleotide set forth in SEQ ID NO:52.

5. To confirm that the polynucleotide set forth in SEQ ID NO:305 is overexpressed in breast tissue, real time PCR was carried out. The first-strand cDNA used in the quantitative real-time PCR was synthesized from 20 μg of total RNA that was treated with DNase I (Amplification Grade, Gibco BRL Life Technology, Gaithersburg, MD), using Superscript Reverse Transcriptase (RT) (Gibco BRL Life Technology, Gaithersburg, MD). Real-time PCR was performed with a GeneAmp™ 5700 sequence detection system (PE Biosystems, Foster City, CA). The 5700 system uses SYBR™ green, a fluorescent dye that only intercalates into double stranded DNA, and a set of gene-specific forward and reverse primers. The increase in fluorescence was monitored during the whole amplification process. The optimal concentration of primers was determined using a checkerboard approach and a pool of cDNAs from tumors was used in this process. The PCR reaction was performed in 25 μl volumes that included 2.5 μl of SYBR green buffer, 2 μl of cDNA template and 2.5 μl each of the forward and reverse primers for the gene of interest. The cDNAs used for quantitative real time PCR reactions were diluted 1:10 for each gene of interest and 1:100 for the β-actin control. Levels of mRNA were expressed relative to β-actin.

6. The real time PCR results showed that mRNA expression for B854P was present at a 10-100 fold higher level in approximately 30% of breast tumors as compared to a panel of normal tissues (see Figure 2).

7. Immunohistochemical (IHC) analysis was performed to determine B854P protein expression in breast cancer and normal tissues. IHC analysis was performed with the affinity purified anti-B854P polyclonal antibodies generated to the peptide sequences 1-4 shown below.

(1) VIQDRKESLKDKLKQDTTQKRRW, amino acid residues 260-282 of the B854P protein as set forth in SEQ ID NO:307.

(2) GHKEFYPVKEFEVYHKLMEKYPC, amino acid residues 56-78 of the B854P protein as set forth in SEQ ID NO:307.

(3) GRGLVTLDGSKWKKHRQIVKPGF, amino acid residues 122-144 of the B854P protein as set forth in SEQ ID NO:307.

(4) HQGSIQLDSTLDSYLKAVFNLSKI, amino acid residues 198-221 of the B854P protein as set forth in SEQ ID NO:307.

8. To generate the polyclonal antibodies, 400 micrograms of the combined peptides that were conjugated to KLH was combined with 100 micrograms of muramyldipeptide (MDP). Equal volume of Incomplete Freund's Adjuvant (IFA) was added and then mixed. Every four weeks animals were boosted with 100 micrograms of antigen mixed with an equal volume of IFA. Seven days following each boost the animal was bled. Sera was generated by incubating the blood at 4° C for 12-24 hours followed by centrifugation.

9. The polyclonal antisera was characterized as follows. Ninety-six well plates were coated with antigen by incubating with 50 microliters (typically 1 microgram) at 4° C for 20 hours. Two hundred and fifty microliters of BSA blocking buffer was added to the wells and incubated at room temperature (RT) for 2 hours. Plates were washed 6 times with PBS/0.01% Tween. Rabbit sera was diluted in PBS. Fifty microliters of diluted sera was added to each well and incubated at RT for 30 minutes. Plates were washed as described above before 50 microliters of goat anti-rabbit horse radish peroxidase (HRP) at a 1:10000 dilution was added and incubated at RT for 30 minutes. Plates were washed as described above and 100 microliters of TMB microwell Peroxidase Substrate was added to each well. Following a 15-minute incubation in the dark at room temperature the colorimetric reaction was stopped with 100 microliters of 1N H_2SO_4 and read immediately at 450 nm.

10. For IHC, paraffin-embedded formalin fixed tissue was sliced into 4-micron sections. Steam heat induced epitope retrieval (SHIER) in 0.1 M sodium citrate buffer (pH 6.0) was used for optimal staining conditions. Sections were incubated with 10% serum/PBS for 5 minutes. Primary antibody was added to each section for 25 minutes at indicated concentrations followed by a 25-minute incubation with an anti-rabbit biotinylated

antibody. Endogenous peroxidase activity was blocked by three 1.5-minute incubations with hydrogen peroxidase. The avidin biotin complex/horse radish peroxidase (ABC/HRP) system was used along with DAB chromogen to visualize antigen expression. Slides were counterstained with hematoxylin. As summarized in Table 1 below, 9/10 breast cancer samples were positive for B854P immunoreactivity as were 5/5 normal breast samples. Normal colon showed some reactivity over background whereas thyroid, liver and tonsil were negative.

TABLE 1: SUMMARY OF IHC ANALYSIS OF B854P EXPRESSION

Tissue Type	No. Tissues Positive/No. Tested
Breast Cancer	9/10
Normal Breast	5/5
Liver	0/1
Thyroid	0/1
Tonsil	0/1
Colon	1/1

11. Accordingly, these data confirm, as described in the specification as filed, that B854P has a breast-specific expression profile. Thus, this antigen can be used in any number of diagnostic and therapeutic applications. For example, overexpression of B854P in breast tumor tissue and normal breast tissue, as compared to other normal tissue types, *e.g.*, PBMCs, can be exploited diagnostically. In this case, the presence of metastatic breast tumor cells, for example in a sample taken from the circulation or liver, can be identified and/or confirmed by detecting expression of B854P in the sample, for example using RT-PCR or by a binding assay as described in the specification as filed. It should be noted that expression of the B854P protein in normal breast tissue does not preclude its use as a diagnostic indicator nor is it a concern with regard to therapeutic applications.

12. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false

statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Davin Dillon, Ph.D.

July 12, 2004
Date